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EXAMINER	
PAK, YONG D	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/572,711	BOGOSIAN ET AL.	
	Examiner	Art Unit	
	YONG D. PAK	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 June 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-5,8-12,14,15,42-46 and 49* is/are pending in the application.

4a) Of the above claim(s) *44-46* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-5,8-12,14,15,42,43 and 49* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This application is a 371 of PCT/US04/31224.

The amendment filed on June 20, 2009, amending claims 1, 3-5, 12, 43, and 45-46, canceling claims 13, 16-18, and 47-48 and adding claim 49, has been entered. No new matter has been entered.

Claims 1-5, 8-12, 14-15, 42-46 and 49 are pending. Claims 44-46 are withdrawn. Claims 1-5, 8-12, 14-15, 42-43 and 49 are under consideration.

Response to Arguments

Applicant's amendment and arguments filed on June 30, 2009, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

In view of the amendment of claims 3 and 12, the rejection of claims 3-4 and 12 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention has been **withdrawn**.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-3, 8-12, 14-15, 42-43, and 49 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1-3, 8-12, 14-15, 42-43, and 49 are drawn to a method of reducing the incorporation of non-standard amino acids into a heterologous protein in a microorganism by co-expressing in said microorganism said heterologous protein and a (A) non-standard amino acid degrading protein or a non-standard amino acid degrading protein or microbial origin, (B) glutamate dehydrogenase, or (C) an *E. coli* glutamate dehydrogenase having a leucine at the amino acid position that corresponds with amino acid 92 of a wild type glutamate dehydrogenase. It is noted that MPEP 2111.01 states that "[d]uring examination, the claims must be interpreted as broadly as their terms reasonably allow." In this case, the an *E. coli* glutamate dehydrogenase having a leucine at the amino acid position that corresponds with amino acid 92 of a wild type glutamate dehydrogenase is not limited to only the substitution at position 92 since the transitional phrase "having" does not create a presumption that the body of the claim is

closed (See MPEP 2111.03). Therefore, while the variant glutamate dehydrogenase comprises the recited substitution, the same variant glutamate dehydrogenase can comprise any amino acids in any other positions. Therefore, the claims are encompass a method of using (A) any or all non-standard amino acid degrading protein isolated from any or all source or any or all microbial sournce, including any or all mutants, recombinants and variants thereof, (B) any or all glutamate dehydrogenases, isolated from any or all source, including any or all mutants, recombinants and variants thereof, or (C) any or all variants of a glutamate dehydrogenase from *E. colil* comprising a leucine residue at position 92 and any other amino acids at any other position. Therefore, the claims are drawn to a method of using a genus of polypeptides having non-standard amino acid degrading activity, but having unknown structure.

In *University of Calfornia v. Eli Lilly & Co.,* 43 USPQZd 1938, the Court of Appeals for the Federal Circuit has held that "A written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula, (or) chemical name,' of the claimed subject matter sufficient to distinguish it from other materials". As indicated in MPEP 2163, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that Applicant was in

possession of the claimed genus. In addition, MPEP 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

The recitation of "non-standard amino acid degrading" and "glutamate dehydrogenase" fails to provide a sufficient description of the claimed genus of proteins as it merely describes the functional features of the genus without providing any definition of the structural features of the species within the genus. The CAFC in UC California v. Eli Lilly, (43 USPQ2d 1398) stated that: "in claims to genetic material, however a generic statement such as 'vertebrate insulin cDNA' or 'mammalian insulin cDNA,' without more, is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by function. It does not specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus." Similarly with the claimed genus of "non-standard amino acid degrading" and "glutamate dehydrogenase" proteins, the functional definition of the genus does not provide any structural information commonly possessed by members of the genus which distinguish the protein species within the genus from other proteins such that one can visualize or recognize the identity of the members of the genus.

Therefore, in the instant case, the claim is drawn to a method of using a genus of polypeptides having non-standard amino acid degrading activity, but having unknown structure. The specification only describes a method of reducing the incorporation of non-standard amino acids of a heterologous polypeptide produced by a microorganism by transforming into said microorganism a vector comprising said heterologous polypeptide and the glutamate dehydrogenase of SEQ ID NO:2 or 4. While MPEP 2163 acknowledges that in certain situations "one species adequately supports a genus," it also acknowledges that "[f]or inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus." In view of the widely variant species encompassed by the genus, this one example is not enough and does not constitute a representative number of species to describe the whole genus of any or all variants, recombinant and mutants of any or all polypeptides having non-standard amino acid degrading activity, including any or all variants, recombinants and mutants thereof, and there is no evidence on the record of the relationship between the structure of the non-standard amino acid degrading protein/glutamate dehydrogenase of SEQ ID NO:2 or 4 and the structure of any or all recombinant, variant and mutant of any or all polypeptides having non-standard amino acid degrading activity. Therefore, the specification fails to describe a representative species of the genus comprising any or all polypeptides having non-standard amino acid degrading activity, including any or all variants, recombinants and mutants thereof.

Given this lack of additional representative species as encompassed by the claims, applicants have failed to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize applicants were in possession of the claimed invention.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

In response to the previous Office Action, applicants have traversed the above rejection. Applicants should note that the rejection has been amended in light of the amendment of the claims.

Applicants argue that the facts of the UC v Lilly cases are distinguishable over the instant case because *E. coli* GDH enzymes were known in the art. Examiner respectfully disagrees. The claims are not limited to a method of using wild type E. coli GDH known in the art, but a method of using (A) any or all non-standard amino acid degrading protein isolated from any or all source or any or all microbial source, including any or all mutants, recombinants and variants thereof, (B) any or all glutamate dehydrogenases, isolated from any or all source, including any or all mutants, recombinants and variants thereof, or (C) any or all variants of a glutamate dehydrogenase from *E. colil* comprising a leucine residue at position 92 and any other amino acids at any other position. Therefore, similarly to the Lilly case, the functional recitation (non-standard degrading protein) fails to provide a sufficient description of the claimed genus of proteins as it merely describes the functional features of the genus

without providing any definition of the structural features of the species within the genus. Said functional limitation does not specifically define any of the proteins that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus.

Applicants also argue that since the specification provides various examples of NSAADPs, the claims meet the written description requirement. Examiner respectfully disagrees. As discussed above, the claims are not limited to a method of using specific wild type or specific mutant NSAADPs but a method of using (A) any or all non-standard amino acid degrading protein isolated from any or all source or any or all microbial source, including any or all mutants, recombinants and variants thereof, (B) any or all glutamate dehydrogenases, isolated from any or all source, including any or all mutants, recombinants and variants thereof, or (C) any or all variants of a glutamate dehydrogenase from *E. coli* comprising a leucine residue at position 92 and any other amino acids at any other position. The genus comprising NSAADPs having unknown structure used in the claimed method does not possess any common attributes other than having the recited activity. Therefore, the specification lacks description of a representative number of species to describe the whole genus. As discussed in the written description guidelines, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant,

identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. A representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. Satisfactory disclosure of a representative number depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only a few species within the genus. In the instant case the claimed genera of the claims includes species which are widely variant in structure. The claims are drawn to structurally diverse species as it encompasses NSAADPs having unknown structure. As such, the description of solely functional features present in all members of the genus is insufficient to be representative of the attributes and features of the entire genus.

Hence the rejection is maintained.

Claims 1-3, 8-12, 14-15, 42-43, and 49 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of reducing the incorporation of non-standard amino acids of a heterologous polypeptide produced by a

microorganism by transforming into said microorganism a vector comprising said heterologous polypeptide and the glutamate dehydrogenase of SEQ ID NO:2 or 4, does not reasonably provide enablement for a method of using any or all polypeptides having non-standard amino acid degrading activity, but having unknown structure The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required are summarized in In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir. 1988). They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

Claims 1-3, 8-12, 14-15, 42-43, and 49 are drawn to a method of reducing the incorporation of non-standard amino acids into a heterologous protein in a microorganism by co-expressing in said microorganism said heterologous protein and a (A) non-standard amino acid degrading protein or a non-standard amino acid degrading protein or microbial origin, (B) glutamate dehydrogenase, or (C) an *E. coli* glutamate dehydrogenase having a leucine at the amino acid position that corresponds with amino acid 92 of a wild type glutamate dehydrogenase.

The breadth of the claims.

It is noted that MPEP 2111.01 states that "[d]uring examination, the claims must be interpreted as broadly as their terms reasonably allow." In this case, the an *E. coli* glutamate dehydrogenase having a leucine at the amino acid position that corresponds with amino acid 92 of a wild type glutamate dehydrogenase is not limited to only the substitution at position 92 since the transitional phrase "having" does not create a presumption that the body of the claim is closed (See MPEP 2111.03). Therefore, while the variant glutamate dehydrogenase comprises the recited substitution, the same variant glutamate dehydrogenase can comprise any amino acids in any other positions. Therefore, the claims are encompass a method of using (A) any or all non-standard amino acid degrading protein isolated from any or all source or any or all microbial sournce, including any or all mutants, recombinants and variants thereof, (B) any or all glutamate dehydrogenases, isolated from any or all source, including any or all mutants, recombinants and variants thereof, or (C) any or all variants of a glutamate dehydrogenase from *E. colil* comprising a leucine residue at position 92 and any other amino acids at any other position. Therefore, the claims are drawn to a method of using polypeptides having non-standard amino acid degrading activity, but having unknown structure. Therefore, the breadth of these claims is much larger than the scope enabled by the specification.

The scope of the claim is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of a method of using any or all polypeptides having non-standard amino acid degrading activity, but having unknown structure. In the instant case, the specification enables only a method of reducing the

incorporation of non-standard amino acids of a heterologous polypeptide produced by a microorganism by transforming into said microorganism a vector comprising said heterologous polypeptide and the glutamate dehydrogenase of SEQ ID NO:2 or 4.

The state of prior art, the relative skill of those in the art, and predictability or unpredictability of the art.

Since the amino acid sequence of the protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. In addition, the art does not provide any teaching or guidance as to (1) which amino acids within a non-standard amino acid degrading protein/glutamate dehydrogenase can be modified and which ones are conserved such that one of skill in the art can make the recited polypeptides having the same biological activity as that of the polypeptide of SEQ ID NO:2 or 4, (2) which segments of SEQ ID NO:2 or 4 are essential for activity, and (3) the general tolerance of non-standard amino acid degrading protein/glutamate dehydrogenase to structural modifications and the extent of such tolerance. The art clearly teaches that changes in a protein's amino acid sequence to obtain the desired activity without any guidance/knowledge as to which amino acids in a protein are required for that activity is highly unpredictable. At the time of the invention, there was a high level of unpredictability associated with altering a polypeptide sequence with an

expectation that the polypeptide will maintain the desired activity. For example, Branden et al. (Introduction to Protein Structure, Garland Publishing Inc., New York, page 247, 1991) teach that (1) protein engineers are frequently surprised by the range of effects caused by single mutations that they hoped would change only one specific and simple property in enzymes, (2) the often surprising results obtained by experiments where single mutations are made reveal how little is known about the rules of protein stability, and (3) the difficulties in designing de novo stable proteins with specific functions.

The amount of direction or guidance presented and the existence of working examples.

The specification discloses a method of reducing the incorporation of non-standard amino acids of a heterologous polypeptide produced by a microorganism by transforming into said microorganism a vector comprising said heterologous polypeptide and the glutamate dehydrogenase of SEQ ID NO:2 or 4. However, the speciation fails to provide any information as to (1) specific substrates associated with any non-standard amino acid degrading protein/glutamate dehydrogenase isolated from any source, including variants, mutants and recombinants thereof, (2) structural elements required in a polypeptide having non-standard amino acid degrading protein/glutamate dehydrogenase activity, or (3) which are the structural elements in a non-standard amino acid degrading protein/glutamate dehydrogenase that are essential to display non-standard amino acid degrading protein/glutamate dehydrogenase activity. No correlation between structure and function of having non-standard amino acid degrading protein/glutamate dehydrogenase activity has been presented. There is no information

or guidance as to which amino acid residues in the polypeptides of SEQ ID NO:2 or 4 can be modified and which ones are to be conserved to create a polypeptide displaying the same activity as that of the polypeptides of SEQ ID NO:2 or 4.

The quantity of experimentation required to practice the claimed invention based on the teachings of the specification.

While enzyme isolation techniques, recombinant and mutagenesis techniques were known in the art at the time of the invention, e.g. hybridization or mutagenesis, and it is routine in the art to screen for multiple substitutions or multiple modifications as encompassed by the instant claims, the specific amino acid positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions. Furthermore, it is not routine in the art to create variants of polynucleotides encoding polypeptides having the activity recited without any knowledge as to the structural features which would correlate with that activity.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including a method of using any or all polypeptides having non-standard amino acid degrading activity, including variants, mutants, recombinants and fragments thereof. The scope of the claims must bear a reasonable

correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of any or all mutants, variants and recombinants of any or all polypeptides having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

In response to the previous Office Action, applicants have traversed the above rejection. Applicants should note that the rejection has been amended in light of the amendment of the claims.

Applicants also argue that since the specification provides various examples of NSAADPs, the claims meet the enablement requirement. Examiner respectfully disagrees. As discussed above, the claims are not limited to a method of using specific wild type or specific mutant NSAADPs but a method of using (A) any or all non-standard amino acid degrading protein isolated from any or all source or any or all microbial source, including any or all mutants, recombinants and variants thereof, (B) any or all glutamate dehydrogenases, isolated from any or all source, including any or all mutants, recombinants and variants thereof, or (C) any or all variants of a glutamate dehydrogenase from *E. coli* comprising a leucine residue at position 92 and any other amino acids at any other position. As discussed above, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a specific knowledge of and guidance with regard to which specific amino acids in the protein's sequence, can be modified such that the modified polypeptide continues

to have said claimed activity. It is this specific guidance that applicants do not provide. Without specific guidance, those skilled in the art will be subjected to undue experimentation of making and testing each of the enormously large number of mutants that results from such experimentation. While the art may teach in general the structure of GDH or GDH having NSAADP activity, conserved amino acid sequences, and etc, such teachings will not reduce the burden of undue experimentation on those of ordinary skill in the art. Hence the rejection is maintained.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-2, 8-11, 14-15, 42-43, and 49 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wang et al., Bogosian et al. and Fenton et al.

Claims 1-2, 8-11, 14-15, 42-43, and 49 are drawn to a method of reducing the incorporation of non-standard amino acid, norleucine, into a bovine somatotropin in a microorganism by co-expressing in said microorganism said somatotropin and a glutamate dehydrogenase having non-standard amino acid degrading protein activity.

Wang et al. (Eur J Biochem. 2001 Nov;268(22):5791-9 – form PTO-892) discloses a mutant glutamate dehydrogenase isolated from *Clostridium symbiosum*, wherein said mutant has a K89L mutation and said mutant has increased activity for degrading norleucine (abstract, Table 4 on page 5796).

Bogosian et al. (US Patent No. 5.932,439 – form PTO-892) discloses expression and production of bovine somatotropin (Column 8, lines 27-51).

The difference between the above references and the instant invention is that the above references do not teach a method of using the mutant of Wang et al. in a method of reducing the incorporation of norleucine in bovine somatroponin.

However, it is well known in the art that that production of heterologous proteins in *E. coli* is hampered by incorporation of non-standard amino acids such as norleucine (Fenton et al. – US Patent No. 5,599,690 – form PTO-892, Column 1).

Therefore, combining the teachings of Wang et al., Bogosian et al. and Fenton et al., it would have been obvious to one having ordinary skill in the art at the time the

claimed invention was made to reduce the incorporation of norleucine in production of heterologous proteins, such as bovine somatotropin, in *E. coli*, by transforming *E. coli* with a vector comprising said heterologous protein or bovine somatropin of Bogosian et al. and the mutant of Fenton et al. or two vectors comprising each of the proteins.

One of ordinary skill in the art would have been motivated to combine the above references in order to reduce incorporation of norleucine when producing heterologous proteins in *E. coli*. One of ordinary skill in the art would have had a reasonable expectation of success since Wang et al. teaches a mutant enzyme which degrades norleucine and Bogosian et al. teaches expression of bovine somatotropin.

Therefore, the above references render claims 1-2, 8-11, 14-15, 42-43, and 49 *prima facie* obvious.

In response to the previous Office Action, applicants have traversed the above rejection. Applicants have provided arguments to both of the 103(a) rejections together.

Applicants argue that since (1) the mutant GDH taught by Wang degrades standard amino acid methionine in addition to norleucine and (2) such mutants would have been expected to impede expression of heterologous proteins, skilled artisans would not have been motivated to overexpress the mutants of Wang et al. Examiner respectfully disagrees. (1) Wang et al. compares activity of GDH mutants towards glutamate and several other monocarboxylic amino acids, including Met and Nle (norleucine). Table 2 on page 5795 discloses that at some pH levels, activity towards Nle is much greater than Met. For example, at pH 8.0, the triple mutant KSA/LAG has no activity towards glutamate, negligible activities towards Leu and Ile, and three times

the rate of degrading Nle than Met. Therefore, one having ordinary skill in the art would expect a much greater degradation of Nle than Met and would have been motivated to use said mutant to degrade/reduce incorporation of Nle in heterologous proteins. (2) Since the instant claims are drawn to a method of reducing the incorporation of norleucine into a heterologouos protein and does not recite any limitation on degree/efficiency of expression of said heterologous protein and degradation of Nle is much greater than Met, one having ordinary skill in the art would have been motivated to co-express the mutant(s) of Wang et al. with a heterologous protein in order to decrease incorporation of norleucine.

Applicants also argue that the motivation to use wild type and mutant forms of Wang et al. is further eroded by unpredictability inherent to extending *in vitro* enzymatic observations to *in vivo* conditions since the claimed invention provided unexpected results: (1) expression of a heterolgous protein is surprising in view of the negative effects on protein translation that would have been expected to occur in attempting to overexpress NSAADPs and (2) Wang et al. discloses that wildtype *C. symbiosum* GHD lacks norleucing degrading activity while wild type *E. coli* GHD reduces norleucine incorporation in heterolgously expressed protein. Examiner respectfully disagrees. Obviousness does not require absolute predictability, but a reasonable expectation of success (see MPEP 2143.02). (1) The claims are not drawn to a method of producing a heterologous protein, but the instant claims are drawn to a method of reducing the incorporation of norleucine into a heterologouos protein. The claims do not recite any limitation on the efficiency or yield of the expression of the heterologous protein. Since

it was well known in the art that that production of heterologous proteins in *E. coli* is hampered by incorporation of non-standard amino acids such as norleucine and Wang et al. discloses a mutant GDH having no or negligible activity towards glutamate and greater activity towards norleucine than Met or other monocarboxylic acids, one having ordinary skill in the art would have been motivated to co-express the mutant of Wang et al. with a heterologous protein in order to decrease incorporation of norleucine. (2) The rejection is not based on using wildtype E. coli GDH, but a mutant E. coli comprising a mutation at a position corresponding to position 89 of the wildtype *C. symbiosum* of Wang et al. Therefore, comparison of norleucine acitivity between wildtype *C. symbisoum* GDH and E. coli GDH are moot.

Hence the rejection is maintained.

Claims 3-5 and 12 rejected under 35 U.S.C. 103(a) as being unpatentable over Wang et al., Bogosian et al. and Fenton et al. as applied to claims 1-2, 8-11, 14-15, 42-43, and 49 above, and further in view of Rice et al.

Claims 3-5 and 12 are drawn to a method of reducing the incorporation of non-standard amino acids into a bovine somatropin in a microorganism by co-expressing in said microorganism said somatropin and a non-standard amino acid degrading protein/glutamate dehydrogenase variant (K92L) comprising the amino acid sequence of ID NO:4 which is encoded by a sequence of SEQ ID NO: 3.

As discussed, above, it would have been obvious to one having ordinary skill in the art to reduce the incorporation of non-standard amino acid, norleucine, into a bovine somatotropin in a microorganism by co-expressing in said microorganism said somatotropin and a non-standard amino acid degrading protein/glutamate dehydrogenase variant (K92L). Further, Wang et al. teaches that lysine 89 (which corresponds to lysine 92 in E. coli glutamate dehydrogenase) is in the substrate binding site (page 5792).

The difference between the combined teachings of Wang et al., Bogosian et al. and Fenton et al. is that the reference to not each a non-standard amino acid degrading protein/glutamate dehydrogenase variant (K92L) comprising the amino acid sequence of ID NO:4 which is encoded by a sequence of SEQ ID NO: 3.

However, Rice et al. (FEMS Microbiol Rev. 1996 May;18(2-3):105-17 - form PTO-892) discloses a wildtype glutamate dehydrogenase isolated from *E. coli* (Figure 1 on page 107). Lysine at position 92 of wildtype E. coli glutamate dehydrogenase corresponds to the lysine residue at 89 of the glutamate dehydrogenase of Wang et al.

Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to make a K92L mutation in an *E. coli* glutamate dehydrogenase in order to make an enzyme that degrades non-standard amino acids and use said mutant enzyme to reduce incorporation of heterologous proteins in *E. coli*.

One of ordinary skill in the art would have been motivated to use a mutant *E. coli* non-standard amino acid degrading protein/glutamate dehydrogenase since *E. coli* is

used very often to express and produce heterolous proteins. One of ordinary skill in the art would have had a reasonable expectation of success since lysine 92(E. coli)/lysine 89 (*C. symbiosum*) is in the substrate binding pocket.

Therefore, the above references render claims 3-5 and 12 *prima facie* obvious.

In response to the previous Office Action, applicants have traversed the above rejection. Since applicants have provided arguments to both of the 103(a) rejections together, see above for Examiner's rebuttal of applicant's arguments.

Conclusion

Claims 1-5, 8-12, 14-15, 42-43, and 49 are rejected.

No claim is allowed.

Conclusion

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the

shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Yong Pak whose telephone number is 571-272-0935. The examiner can normally be reached 6:30 A.M. to 5:00 P.M. Monday through Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll free).

/Yong D Pak/
Primary Examiner, Art Unit 1652